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# PHOSPHOLIPID FATTY ACID COMPOSITION AND ARACHIDONIC ACID UPTAKE AND METABOLISM BY THE CICADA *TIBICEN DEALBATUS* (HOMOPTERA: CICADIDAE)

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**Abstract**—1. The phospholipid fatty acid compositions of whole animals and selected tissues from adult males and females of the cicada *Tibicen dealbatus* were analyzed.

2. The major components were C16 and C18 saturated and unsaturated acids nearly ubiquitous in most animals.

3. In addition, small quantities of other monoenoic and dieneoic acids, linolenic acid (18:3*n*-3), a hydroxy fatty acid (2-OH-20:0) and two prostaglandin precursor fatty acids, 20:3*n*-6 and arachidonic acid (20:4*n*-6) were present.

4. The per cent composition of these fatty acids varied considerably between sexes and among the various tissues examined.

5. Injected radioactive arachidonic acid was converted into at least three prostaglandins, $PGD_2$, $PGE_2$, and $PGF_{2\alpha}$, as well as into other more polar oxygenated derivatives.

6. The data are discussed in terms of a hypothesis linking eicosanoids and thermoregulation in this cicada.

## INTRODUCTION

Many species of cicada are diurnally active under conditions of high ambient temperature ($T_a$) and radiative heat loadings that can drive their body temperature ($T_b$) to deleteriously high levels. They respond to this threat by a combination of behavioral mechanisms (Heath and Wilkin, 1970; Heath *et al.*, 1972; Hastings, 1989) and evaporative cooling (Toolson, 1987). The high water loss rates (WLRs) required for effective evaporative cooling result from a combination of an inherently high cuticular permeability to water (Toolson, 1984, 1987; Hadley *et al.*, 1989) and from activation of an energy-dependent process that can increase transcuticular water flux (TWF) rates by nearly an order of magnitude within 1–2 min (Toolson and Hadley, 1987; Hadley *et al.*, 1989). This process, known only in cicadas, is activated at species-specific set-point temperatures ($T_{sp}$) and is regulated by as yet unknown mechanisms.

For some mammals and non-insect invertebrates, certain prostaglandins (PGs) have been shown to stimulate hyperthermia (Stanley-Samuelson, 1987). Vertebrate examples include behavioral hyperthermia in frogs (Casterlin and Reynolds, 1977a; Myhre *et al.*, 1977), a lizard (Bernheim and Kluger, 1976) and several fish (Reynolds *et al.*, 1976). In these studies it was shown that injection of pathogenic bacteria or chemical pyrogens caused the animals to behaviorally raise $T_b$ by selecting a warmer zone within a broad temperature gradient.

Similar experiments with several invertebrate species showed that injection of pathogenic bacteria or pharmacological doses of PGs resulted in behavioral hyperthermia. Examples include the freshwater crayfish *Cambarus bartoini* (Casterlin and Reynolds, 1977b, 1978), and three marine species, the American lobster *Homarus americanus*, the pink shrimp *Penaneus duorarum* and the horseshoe crab *Limulus polyphemus* (Casterlin and Reynolds, 1979). In addition to these aquatic invertebrates, two terrestrial invertebrates, the scorpions *Buthus occitanus* and *Androctonus australis* developed extraordinarily high hyperthermia (>10°C) following injections of μg quantities of $PGE_1$ (Cabanac and LeGuelte, 1980). These scorpions are North African species that are probably capable of tolerating very high body temperatures for at least brief periods. In our judgement, some of the conclusions from the above papers of hyperthermia in invertebrates are not completely supported by the data; however, taken as a group, they still strongly suggest that PGs may be important mediators of thermoregulation in a wide variety of invertebrate taxa (Stanley-Samuelson, 1987).

Preliminary experiments in our laboratories on the effects of exogenous PGs and known PG biosynthesis inhibitors on $T_b$ in *Tibicen dealbatus* clearly suggested that PGs may be involved in TWF rates in these cicadas (Toolson, Howard and Stanley-Samuelson, unpublished observations). Previous studies of thermobiology of invertebrates have not documented the presence of endogenous PGs or related eicosanoids,

thereby leaving open the possibility that the hyperthermic response to exogenous PGs represents a pharmacological rather than a normal physiological response. Consequently, we have undertaken biochemical studies to demonstrate that the essential C20 polyunsaturated fatty acid (PUFA) prostaglandin precursors are endogeneous components of *T. dealbatus* tissues and that this species can convert these compounds into PGs. In this paper we have characterized whole organisms and selected tissue phospholipid (PL) fatty acid profiles, demonstrated that all PUFA precursors necessary for the biosynthesis of 1- and 2-series prostaglandins are present, and shown that radioactive arachidonic acid is converted into at least three PGs *in vivo*.

## MATERIALS AND METHODS

### *Insects*

Adult male and female *T. dealbatus* were collected from sycamore trees (*Platanus* sp.) on the University of New Mexico campus during July and August, 1989. They were shipped immediately via air in chilled containers to the University of Nebraska. As soon as the insects were rceived (within 24 hr of collection) they were placed on sycamore cuttings, allowed to feed and drink for 24–30 hr, and then used for experiments.

### *Gas chromatography (GC) of fatty acids*

Lipid extraction, separation of lipid fractions, formation of fatty acid methyl esters (FAMEs) and GC analysis closely followed methods described by Stanley-Samuelson and Dadd (1983, 1984). Briefly, either whole animals were processed or selected tissues (heads, thoracic muscles, dorsal abdominal glands, malpighian tubules, male guts, and ovaries) were isolated and placed in tissue grinders containing chloroform:methanol (2:1, v:v). Fifty microliters of 2% butylated hydroxytoluene (BHT) in chloroform were added to each tube to reduce autooxidation of PUFAs. Total lipids were extracted using the method of Bligh and Dyer (1959). Phospholipids were isolated by thin layer chromatography and transmethylated by refluxing in acidified methanol for 30 min (Stanley-Samuelson and Dadd, 1983, 1984). Fatty acid methyl esters were then chromatographed isothermally at 190°C using a Hewlett-Packard HP5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a SP-2330 capillary columm (0.25 mm × 30 M, 0.2 $\mu$m film thickness, Supelco, Bellefonte, PA), a flame ionization detector (FID), and a HP-3396A recording integrator. A split ratio of 45:1 was used and separations were carried out with $He_2$ as the carrier gas at a flow rate of 0.6 ml/min. Fatty acid methyl ester components were tentatively identified by comparisons of retention times with authentic FAME standards (Sigma Chemical Company, St Louis, MO). The number of animals or tissues used for each analysis are listed in Table 1.

### *Gas chromatography–mass spectrometry of FAMEs*

Analyses were conducted by capillary GC-electron impact mass spectrometry (EI-MS) using a Hewlett–Packard 5790 GC equipped with a SP-2330 column of the same specifications described above. The GC was interfaced to a Hewlett–Packard 5970 mass selective detector operated at 70 eV. A 45 sec splitless injection was used, and all GC runs utilized temperature programming from 170–200°C at 1°C/min, with an initial 1 min hold period. Ultrapure $He_2$ was the carrier gas with a flow rate of 1 ml/min. Retention times and total ion EI-mass spectra of even carbon number FAMEs were compared to FAME standards obtained from Sigma Chemical Company. Hydroxy FAMEs were characterized by comparison to published EI-mass spectra (McCloskey, 1970).

### *Biosynthesis of prostaglandins*

Tritiated arachidonic acid ($^3$H-5,6,8,9,11,12,14,15-20:4*n*-6, spec. act. 180–240 mCi/mmol, New England Nuclear, Cambridge, MA) was purified by elution with toluene from a silica gel mini-column (Bio-sil A, 100–200 mesh, Bio-Rad, Richmond, CA), then made up in 100% EtOH to a spec. act. of 1 $\mu$Ci/$\mu$l. One $\mu$Ci of tritiated 20:4*n*-6 was injected into individual adults between the second and third sclerites. After 5 min unmetabolized 20:4*n*-6 and oxygenated metabolites were extracted three times from individual whole animals by homogenization in 5 ml of ethyl acetate acidified with 1 ml of 0.1 N HCl. The pooled organic phases from each extraction were evaporated under $N_2$. Extracts were then separated into $PGD_2$, $PGE_2$, $PGF_{2\alpha}$ and more polar metabolites by silica gel thin layer chromatography (TLC). Plates were developed in the saturated organic layer formed by mixing ethyl acetate:isooctane:water:acetic acid (55:25:50:10, v:v) (Hurst *et al.*, 1987). Standard PG mixtures were added to the origin of each sample as carriers and individual PG standards (Biomol Research Laboratories, Plymouth Meeting, PA) were spotted in separate lanes for identification. Individual fractions were visualized by exposing the TLC plates to $I_2$ vapors and collected by scraping into liquid scintillation vials. Radioactivity was assayed by liquid scintillation counting using a Pharmacia-LKB 1209 LS counter (Gaithersburg, MD) equipped with chemiluminescence discrimination at 70% efficiency for tritium.

Table 1. Phospholipid fatty acid composition of whole animal and selected tissues of *Tibicen dealbatus*

| | Per cent of total fatty acids | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fatty | Head | | Thoracic muscles | | Abdominal dorsal glands | | Malpighian tubules | | Guts | | Whole organisms | |
| acid | M* | F† | M | F | M | F | M | F | (male only) | Ovaries | M | F |
| 14:0 | 0.3 | 0.1 | 0.2 | 0.2 | 0.4 | 0.7 | <0.1‡ | 1.5 | 0.2 | 0.4 | 0.1 | 0.2 |
| 16:0 | 6.1 | 5.4 | 11.3 | 5.7 | 4.2 | 6.6 | 6.1 | 9.1 | 2.6 | 4.1 | 3.0 | 6.8 |
| 16:1 | 1.7 | 1.1 | 1.8 | 1.5 | 1.5 | 0.8 | 1.5 | 2.3 | 0.9 | 1.0 | 1.0 | 1.6 |
| 18:0 | 12.4 | 5.4 | 3.5 | 3.7 | 11.2 | 10.0 | 11.4 | 14.6 | 11.4 | 13.2 | 11.7 | 9.2 |
| 18:1 | 41.2 | 29.1 | 23.1 | 26.1 | 44.5 | 41.0 | 49.7 | 45.4 | 47.4 | 46.6 | 40.3 | 39.5 |
| 18:2 | 34.9 | 55.3 | 53.4 | 58.7 | 33.5 | 37.0 | 30.2 | 22.1 | 34.8 | 28.7 | 37.2 | 39.7 |
| 18:3 (*n*-3) | 0.5 | <0.1 | 0.1 | 0.2 | 0.8 | <0.1 | <0.1 | <0.1 | 0.2 | 0.3 | 0.3 | 0.3 |
| 20:0 | 1.0 | 1.4 | 0.2 | 0.3 | 1.1 | 2.0 | 0.9 | <0.1 | 0.8 | 2.0 | 2.0 | 1.0 |
| 20:1 | 0.2 | <0.1 | 0.1 | 0.1 | 0.3 | <0.1 | <0.1 | <0.1 | 0.2 | 0.3 | 0.1 | 0.2 |
| 20:3 (*n*-6) | 0.1 | <0.1 | 0.2 | 0.2 | 0.1 | <0.1 | <0.1 | <0.1 | 0.1 | <0.1 | 0.1 | 0.1 |
| 20:3 (*n*-3) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 20:4 (*n*-6) | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 22:0 | 0.6 | 0.6 | 0.3 | 0.2 | 0.8 | <0.1 | <0.1 | <0.1 | 0.3 | 0.4 | 1.2 | 0.6 |
| 24:0 | <0.1 | <0.1 | <0.1 | <0.1 | 0.2 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.9 | 0.1 |
| 2-OH-20:0 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| *N*§ | 2 | 2 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 2 | 1 | 1 |

*M = male; †F = Female; ‡<0.1% is regarded as trace levels; §*N* = number of tissues or animals pooled for each analysis.

*Statistical methods and voucher specimens*

Summary statistics and one way analyses of variance were conducted using the PC version of Minitab (Minitab, Reading, MA). Significance tests were conducted at the $\alpha = 0.05$ level. Voucher specimens have been deposited in the research collection of the Department of Entomology, Kansas State University, Manhattan, KS.

## RESULTS

The fatty acid composition of PLs isolated from whole body, heads, thoracic muscles, abdominal dorsal glands, malpighian tubules, male guts, and ovaries are listed in Table 1. In all tissues examined, the major components are 16:0, 18:0, 18:1 and 18:2, as is seen in the majority of insects which have been studied (Stanley-Samuelson *et al.*, 1988). Smaller proportions of 14:0, 16:1, 18:3, 20:0, 20:1, 20:2, 22:0 and 24:0 were also found. Trace quantities of 2-hydroxyeicosanoic acid was detected in all tissues examined. In addition to the fatty acids reported above, two PG precursor PUFAs were present in all tissues examined: homo-gama-linolenic acid (20:3*n*-6), precursor to the 1-series of PGs and arachidonic acid, precursor to the 2-series of PGs.

At the organismal level, there were no sex-related differences in PL fatty acid compositions (Table 1). At the tissue level, however, male heads were richer in 18:0 and 18:1 and poorer in 18:2 than were female heads. Thoracic muscles have a PL fatty acid composition that is different from the other tissues examined (Table 1). Thus, the thoracic muscles have approximately 26% 18:1 whereas most other tissues have about 45% of this acid. Similarly, these muscles contain about 55% of 18:2 whereas most other tissues only contain about 30%. Finally, the thoracic muscles seem to contain only about one-third the proportion of 18:1 as the other tissues.

Adult *T. dealbatus* biosynthesized at least three primary PGs ($PGD_2$, $PGE_2$, $PGF_{2\alpha}$) from injected radioactive arachidonic acid. No sex-related differences in PG biosynthesis were found, so the data were pooled (Table 2). Most of the radioactivity associated with primary PGs was recovered as $PGD_2$, with values for $PGE_2$, $PGF_{2\alpha}$ being *ca* 5% of the level of $PGD_2$. Substantial radioactivity was also recovered as more polar metabolites. These products are most likely lipoxygenase products not yet characterized, and more polar products of PG metabolism.

## DISCUSSION

As in tissues from other vertebrates and invertebrates, 16:0, 16:1, 18:0, 18:1 and 18:2*n*-6 make up the vast majority of *T. dealbatus* PL fatty acids. In addition to these components, we recorded one unusual fatty acid that has either not been detected or, if detected, was not discussed in previous studies. All tissues from *T. dealbatus* contained trace amounts of 2-hydroxy-20:0. Among insects, this acid has only been detected in tissue from the meal worm beetle *Tenebrio molitor* (Howard and Stanley-Samuelson, 1990). Because this component is so newly recognized in insect lipids, we have confirmed its chemical identity by using the mass spectrometric techniques described earlier (Howard and Stanley-Samuelson, 1990). Hydroxy fatty acids are best known from microbes, where they are associated with membrane and cell wall lipids (Harwood and Russell, 1984). They occur in only very low levels in higher organisms, where their biological significance is not well understood. Cicadas possess bacteria in fat body mycetocytes (Chapman, 1982), and it is possible that the hydroxy fatty acid we detected is of bacterial origin.

Table 2. Incorporation of $^3$H-arachidonic acid into prostaglandins by adult *Tibicen dealbatus*

| Prostaglandin | Per cent of counts recovered $\bar{x}$ (SE)* |
|---|---|
| $PGD_2$ | 9.18 (6.17) |
| $PGE_2$ | 0.59 (0.25) |
| $PGF_{2\alpha}$ | 0.50 (0.23) |
| Polar metabolites | 6.15 (2.50) |

*$N$ = 10 cicadas (6♂♂, 4♀♀). One-way ANOVA for each PG by sex indicated no significant differences, so the data were pooled.

Besides this unusual component, we also detected three C20 PUFAs, two of which are PG precursors. These were C20:3*n*-6, C20:3*n*-3 and C20:4*n*-6. Although PGs have been detected in more than a dozen insect species from several diverse orders (Stanley-Samuelson and Loher, 1986), the C20 PUFAs that are necessary for PG biosynthesis have either not been looked for or occurred in such low levels that they were often not detected (Stanley-Samuelson and Dadd, 1983; Stanley-Samuelson *et al.*, 1988). There is considerable variation in amounts of C20 PUFAs among insect orders. They comprise over 20% of total fatty acids in many aquatic insects (Hanson *et al.*, 1985), but typically occur in far lower proportions, often less than 1%, in terrestrial insects (Stanley-Samuelson *et al.*, 1988). The long-chain PUFAs in *T. dealbatus* occurred in small but readily measurable quantities (Table 1), as in other terrestrial insects.

The biological significance of C20 PUFAs undoubtedly extends beyond simply serving as PG precursors. Dadd (1983) showed that in the mosquito *Culex pipiens*, C20 PUFAs were important as structural components of biomembranes, in addition to serving as PG precursors. Multiple roles of C20 PUFAs are well documented among mammalian species (Hansen, 1989).

We did not detect C20:5*n*-3 in *T. dealbatus* even with a rigorous search by GC—MS. Arachidonic acid occurred in only trace quantities, while 20:3*n*-6 was readily detected at several tenths of a per cent of PL fatty acids in all tissues. We take these data to indicate that the 1-series PGs may be quantitatively more important than the 2- and 3-series PGs in *T. dealbatus*. There are other examples of this point: Wakayama *et al.* (1986) showed that the house fly *Musca domestica* synthesizes 1-series PGs far more readily than 2-series PGs. Similarly, many Lepidoptera nutritionally specialize on *n*-3 PUFAs (Dadd, 1983), suggesting that insects that are specialists on *n*-3 PUFAs may favor the production of 3-series PGs.

Within the Homoptera, various taxonomic groups have characteristic fatty acid profiles (Fast, 1970). Most members of Aphidoidea have very high proportions of 12:0 and 14:0, and small proportions of 18:2*n*-6; in some species the two short-chain components made up over 90% of total fatty acids. Coccids

stand out from other Homoptera because of their high proportions of 10:0 and 12:0 components, and very small proportions of longer-chain fatty acids. The fatty acid profiles of members of the Cicadoidea are more diverse. Cercopids and cicadellids have traces of a few per cent of short-chain fatty acid components, higher proportions of 16:0, and substantial proportions of 18:2*n*-6. The one report on a cicada we are aware of (Fast, 1970) shows a fatty acid pattern similar to the whole organism data we report here, with high proportions of 18:1 and 18:2. All of these patterns emerged from the early period of insect fatty acid studies in which C20 PUFAs were not discussed.

We have shown that both sexes of *T. dealbatus* converted radioactive arachidonic acid into PGs. Less than 1% of the label was recovered as material that co-eluted with authentic $PGE_2$, $PGF_{2\alpha}$, and about 9% co-eluted with authentic $PGD_2$. Biosynthesis of $PGE_2$, $PGF_{2\alpha}$ and the presence of the PGs themselves have been observed in several insect species (Stanley-Samuelson and Loher, 1986). Biosynthesis of $PGD_2$ has been observed in only two species so far: the mosquito *Aedes aegypti* (Stanley-Samuelson *et al.*, 1989) and the meal worm beetle *T. molitor* (Howard and Stanley-Samuelson, 1990). About 6% of the injected radioactive C20:4*n*-6 was recovered as more polar products. This material is taken to represent more polar metabolites of various PGs and primary lipoxygenase products. Lipoxygenase products have been detected in the mosquito *C. pipiens* by RIA (Stanley-Samuelson and Dadd, unpublished observations) and lipoxygenase activity was shown in the firebrat *Therobia domestica* (Ragab *et al.*, 1987). Although it remains to be shown, we consider it likely that PG biosynthesis is to be expected in all insect species (Stanley-Samuelson, 1987).

At present, very little is known about the biological significance of PGs and related eicosanoids in insects. Indeed, only one clearly defined role has been identified: PGs are known to be involved in releasing egg-laying behavior in some cricket species (Stanley-Samuelson and Loher, 1986). However, if we broaden attention to the generality of invertebrate phyla, eicosanoids are known to be involved in many different physiological roles. For example, PGs are involved in regulation of ion flux in marine and freshwater bivalves, and certain lipoxygenase products (8-HETE) stimulate oocyte maturation in certain species of starfish. These and many other examples are detailed in the review by Stanley-Samuelson (1987). We surmise from these many examples that the greater significance of PGs in insects will emerge in due course.

While we have not yet shown that PGs play a direct role in regulating water loss during evaporative cooling in cicadas, evidence from other animals suggests that the hypothesis is tenable. Transport of ions is undoubtedly involved in the energy-dependent movement of water through the cuticle during evaporative cooling in cicadas, and the involvement of PGs in water/salt balance physiology in mammals (Braquet *et al.*, 1985) and in marine and freshwater bivalve molluscs (Stanley-Samuelson, 1987) is well established. Moreover, we have demonstrated that injection of exogenous PGs alters $T_b$ in *T. dealbatus* exposed to ambient temperatures high enough to require evaporative cooling, as does inhibition of PG biosynthesis (Toolson, Howard, and Stanley-Samuelson, unpubl. observ.), suggesting that the active regulation of TWF by cicadas is mediated by PGs and, perhaps, other metabolites of arachidonic acid. Our documentation of the presence of endogenous PGs and their precursor PUFAs in *T. dealbatus* and the demonstration that arachidonic acid is oxygenated *in vivo* to produce 2-series PGs provides a basis for future research on the possible role(s) played by PGs and other arachidonic acid derivatives in temperature and water relations of cicadas.

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## REFERENCES

Bernheim H. A. and Kluger M. J. (1976) Fever: affect of drug-induced antipyresis on survival. *Science* **193,** 237–239.

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917.

Braquet P., Garay R. P., Frolich J. C. and Nichosia S. (1985) *Prostaglandins and Membrane Ion Transport. Advances in Ion Transport Regulation.* Raven Press, New York.

Cabanac M. and LeGuelte L. (1980) Temperature regulation and prostaglandin $E_1$ fever in scorpions. *J. Physiol.* **303,** 365–370.

Casterlin M. E. and Reynolds W. W. (1977a) Behavioral fever in anuran amphibian larvae. *Life Sci.* **20,** 593–596.

Casterlin M. E. and Reynolds W. W. (1977b) Behavioral fever in crayfish. *Hydrobiologia* **56,** 99–101.

Casterlin M. E. and Reynolds W. W. (1978) Prostaglandin $E_1$ fever in the crayfish *Cambarus bartoni*. *Pharmac. Biochem. Behav.* **9,** 593–595.

Casterlin M. E. and Reynolds W. W. (1979) Fever induced in marine arthropods by prostaglandin $E_1$. *Life Sci.* **25,** 1601–1604.

Chapman R. F. (1982) *The Insects: Structure and Function.* Harvard University Press.

Dadd R. H. (1983) Essential fatty acids—insects and vertebrates compared. In *Metabolic Aspects of Lipid Nutrition in Insects* (Edited by Mittler T. E. and Dadd R. H.). Westview Press Boulder, CO.

Fast P. A. (1970) Insect lipids. *Prog. Chem. Fats* **11,** 181–242.

Hadley N. F., Toolson E. C. and Quinlan M. (1989) Regional differences in cuticular permiability in the desert cicada *Diceroprocta apache*: implications for evaporative cooling. *J. exp. Biol.* **141,** 219–230.

Hansen H. S. (1989) Linoleic acid—essential fatty acid and eicosanoid precursor. Bondegaard tryk as, Herlev, Denmark.

Hanson B. J., Cummins K. W., Cargill A. S. and Lowry R. R. (1985) Lipid content, fatty acid composition and the effect of diet on fats of aquatic insects. *Comp. Biochem. Physiol.* **80B,** 257–276.

Harwood J. W. and Russell N. J. (1984) *Lipids in Plants and Microbes*. George, Allen & Unwin, London.

Hastings J. (1989) Thermal regulation in the dog day cicada, *Tibicen durbi* (Homoptera: Cicadidae). *Trans. Ky. Acad. Sci.* **50**, 145–149.

Heath J. E. and Wilkin P. J. (1970) Temperature responses of the desert cicada *Diceroprocta apache* (Homoptera: Cicadidae). *Physiol. Zool.* **43**, 145–154.

Heath J. E., Wilkin P. J. and Heath M. S. (1972) Temperature responses of the cactus dodger *Cacama balvata*. *Physiol. Zool.* **45**, 238–246.

Howard R. W. and Stanley-Samuelson D. W. (1990) Arachidonic acid uptake and metabolism by selected tissues of adult *Tenebrio molitor* (L) (Coleoptera: Tenebrionidae) and their phospholipid fatty acid composition. *Annls. Entomol. Soc. Am.* (in press).

Hurst J. S., Flatman S. and McDonald-Gibson R. G. (1987) Thin-layer chromatography (including radio thin-layer chromatography and autoradiography) of prostaglandins and related compounds. In *Prostaglandins and Related Substances, a Practical Approach* (Edited by Benedetto C., McDonald-Gibson R. G., Nigam S. and Slater T. F.), pp. 53–73. IRL Press, Oxford.

McCloskey J. M. (1970) Mass spectrometry of fatty acid derivatives. In *Topics in Lipid Chemistry* (Edited by Gunstone F. E.), Vol 1., pp. 369–440. John Wiley and Sons, New York.

Myhre K., Cabanac M. and Myhre G. (1977) Fever and behavioral temperature regulation in the frog *Rana esculenta*. *Acta physiol. scand.* **101**, 219–229.

Ragab A., Bitsch C., Thomas J. M. F., Bitsch J. and Chap H. (1987) Lipoxygenase conversion of arachidonic acid in males and inseminated females of the firebrat, *Thermobia domestica* (Thysanura). *Insect Biochem.* **17**, 863–870.

Reynolds W. W., Casterlin M. E. and Covert J. B. (1976) Behavioral fever in teleost fishes. *Nature* **259**, 41–42.

Stanley-Samuelson D. W. (1987) Physiological roles of prostaglandins and other eicosanoids in invertebrates. *Biol. Bull.* **173**, 92–109.

Stanley-Samuelson D. W. and Dadd R. H. (1983) Long-chain polyunsaturated fatty acids: patterns of occurrence in insects. *Insect Biochem.* **13**, 549–558.

Stanley-Samuelson D. W. and Dadd R. H. (1984) Polyunsaturated fatty acids in the lipids from adult *Galleria mellonella* reared on diets to which only one unsaturated fatty acid had been added. *Insect Biochem.* **14**, 321–327.

Stanley-Samuelson D. W. and Loher W. (1986) Prostaglandins in insect reproduction. *Ann. Entomol. Soc. Am.* **79**, 841–853.

Stanley-Samuelson D. W., Jurenka R. A., Cripps C., Blomquist G. J. and de Renobales M. (1988) Fatty acids in insects: composition, metabolism, and biological significance. *Arch. Insect Biochem. Physiol.* **9**, 1–33.

Stanley-Samuelson D. W., Mackay M. E. and Blomquist G. J. (1989) Arachidonic acid and prostaglandins in the mosquito, *Aedes aegypti*. In *Host Regulated Developmental Mechanism in Vector Arthropods* (Edited by Borovsky D. and Spielman A.), pp. 179–188. University of Florida, Vera Beach, FL.

Toolson E. C. (1984) Interindividual variation in epicuticular hydrocarbon composition and water loss rates of the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiol. Zool.* **57**, 550–556.

Toolson E. C. (1987) Water profligacy as an adaption to hot deserts: water loss rates and evaporative cooling in the Sonoran Desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). *J. exp. Biol.* **131**, 439–444.

Toolson E. C. and Hadley M. F. (1987) Energy-dependent facilitation of transcuticular water flux contributes to evaporative cooling in the Sonoran Desert cicada *Diceroprocta apache* (Homoptera: Cicadidae). *J. exp. Biol.* **131**, 439–444.

Wakayama E. J., Dillwith J. W. and Blomquist G. J. (1986) Occurrence and metabolism of prostaglandins in the housefly *Musca domestica* (L). *Insect Biochem.* **16**, 895–902.